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=> s scFv and VH and stability
L1 93 SCFV AND VH AND STABILITY

=> s (protein stability)
L2 14682 (PROTEIN STABILITY)

=> s l2 and l1
L3 2 L2 AND L1

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PROCESSING COMPLETED FOR L3
L4 2 DUPLICATE REMOVE L3 (0 DUPLICATES REMOVED)

=> d l3 bib abs 1-2

L3 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2006:676884 CAPLUS
DN 145:138590
TI Ribosome display or mRNA display method combined with selection for
increased stability of the protein, and therapeutic uses of
selected proteins
IN Buchanan, Andrew; Jermutus, Lutz
PA Cambridge Antibody Technology Limited, UK
SO PCT Int. Appl., 79 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2006072773	A1	20060713	WO 2006-GB2	20060105
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,				

CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

GB 2422606	A1	20060802	GB 2006-147	20060105
US 2006183200	A1	20060817	US 2006-326710	20060105
PRAI GB 2005-99	A	20050105		
US 2005-642209P	P	20050105		

AB The invention employs use of display technol. incorporating in vitro translation and covalent (like in mRNA display) or non-covalent (like in ribosome display) linkage between genotype, such as RNA, and the encoded phenotype, such as a polypeptide of interest, to select for polypeptide variants that have improved stability compared with a parent polypeptide and that retain functional activity. The invention provides an RNA expression system, wherein several stability selection pressures are applied during translation and during selecting. A stability selection pressure may affect the ability of a polypeptide to remain in its folded and active state. A stability selection pressure may be a chemical or phys. denaturant, a reducing agent, a protease or enzyme capable of degrading protein. A stability selection pressure may be the use of hydrophobic interaction chromatog. (HIC). In some embodiments the protein of interest is an antibody or a hormone. Demonstrated are construction of ribosome display libraries and selection of EPO (erythropoietin), GM-CSF (colony-stimulating factor 2) and G-CSF (granulocyte colony-stimulating factor) variants with improved stability.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 2 OF 2 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 1995:25323156 BIOTECHNO
TI VI-linker-Vh orientation-dependent expression of single chain Fv containing an engineered disulfide-stabilized bond in the framework regions
AU Luo D.; Mah N.; Krantz M.; Wilde K.; Wishart D.; Zhang Y.; Jacobs F.; Martin L.
CS Research and Development Division, Biomira Inc., 2011-94 Street, Edmonton, Alta. T6N 1H1, Canada.
SO Journal of Biochemistry, (1995), 118/4 (825-831)
CODEN: JOBIAO ISSN: 0021-924X
DT Journal; Article
CY Japan
LA English
SL English
AB Single chain Fv fragments (scFv) derived from an antibody, MAb 174H.64 (Tru-Scint(R)SQ(TM) kit, Biomira), were constructed in both orientations, i.e. Vh-linker-VI and VI-linker-Vh, but only the latter form could be expressed and secreted in the recombinant Pichia pastoris system. The secreted scFv protein showed specific anti-idiotypic binding activity. Additionally, the molecular graphic modeling has been used to identify a possible site for the introduction of an interchain disulfide bond in the framework region of Fv. These Cys-modifications of the sites were done using a method of PCR-mediated mutagenesis. The engineered protein (disulfide-stabilized Fv:dsFv) was expressed and tested for its binding activity. It was found that dsFv was as active as the corresponding scFv and more stable as determined by competitive radioimmunoassay.

=> display history
ENTER (BRIEF), FULL, OR NOFILE:full
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(FILE 'CAPLUS, BIOENG, BIOTECHNO, BIOTECHDS, ES BIOBASE' ENTERED AT
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L1 93 SEA ABB=ON PLU=ON SCFV AND VH AND STABILITY

FILE HOME

FILE CAPLUS

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FILE LAST UPDATED: 20 OCT 2006 <20061020/UP>
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FILE LAST UPDATED: 7 JAN 2004 <20040107/UP>
FILE COVERS 1980 TO 2003.

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/CT AND BASIC INDEX <<<

FILE BIOTECHDS
FILE LAST UPDATED: 8 NOV 2006 <20061108/UP>
FILE COVERS 1982 TO DATE

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FILE ES BIOBASE
FILE LAST UPDATED: 15 NOV 2006 <20061115/UP>
FILE COVERS 1994 TO DATE.

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=> s scFv-Ig and (stability or production)
L5 4 SCFV-IG AND (STABILITY OR PRODUCTION)

=> duplicate remove 15
DUPLICATE PREFERENCE IS 'CAPLUS, BIOTECHNO'
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PROCESSING COMPLETED FOR L5
L6 4 DUPLICATE REMOVE L5 (0 DUPLICATES REMOVED)

=> d 16 bib abs 1-4

L6 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2006:207966 CAPLUS
DN 144:388723
TI Construction and optimization of a CC49-Based scFv- β -lactamase fusion protein for ADEPT
AU Roberge, Martin; Estabrook, Melodie; Basler, Joshua; Chin, Regina; Gualfetti, Pete; Liu, Amy; Wong, Stephanie B.; Rashid, M. Harunur; Graycar, Tom; Babe, Lilia; Schellenberger, Volker
CS a Danisco company, Genencor International, Palo Alto, CA, 94304, USA
SO Protein Engineering, Design & Selection (2006), 19(4), 141-145
CODEN: PEDSBR; ISSN: 1741-0126
PB Oxford University Press
DT Journal
LA English
AB CC49 is a clin. validated antibody with specificity for TAG-72, a carbohydrate epitope that is over-expressed and exposed on a large fraction of solid malignancies. We constructed a single chain fragment (scFv) based on CC49 and fused it to β -lactamase. The first generation fusion protein, TAB2.4, was expressed at low levels in Escherichia coli and significant degradation was observed during prodn. We optimized the scFv domain of TAB2.4 by Combinatorial Consensus Mutagenesis (CCM). An improved variant TAB2.5 was identified that resulted in an almost 4-fold improved expression and 2.5° higher thermostability relative to its parent mol. Soluble TAB2.5 can be manufactured in low-d. E.coli cultures at 120 mg/l. Our studies suggest that CCM is a rapid and efficient method to generate antibody fragments with improved stability and expression. The fusion protein TAB2.5 can be used for antibody directed enzyme prodrug therapy (ADEPT).

RE.CNT 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 4 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 1999:29309382 BIOTECHNO
TI Characterization of scFv-Ig constructs generated from the anti-CD20 mAb 1F5 using linker peptides of varying lengths
AU Shan D.; Press O.W.; Tsu T.T.; Hayden M.S.; Ledbetter J.A.
CS Dr. D. Shan, Univ. of Washington Medical Center, Division of Medical Oncology, Box 356043, Seattle, WA 98195-6043, United States.
E-mail: dmshan@u.washington.edu
SO Journal of Immunology, (01 JUN 1999), 162/11 (6589-6595), 40 reference(s)
CODEN: JOIMA3 ISSN: 0022-1767
DT Journal; Article
CY United States
LA English
SL English
AB The heavy (V(H)) and light (V(L)) chain variable regions of the murine anti-human CD20 mAb 1F5 were cloned, and four single-chain Ab (scFv) molecules were constructed using linker peptides of variable lengths to join the V(H) and V(L) domains. Three constructs were engineered using linker peptides of 15, 10, and 5 aa residues consisting of (GGGGS).sub.3, (GGGGS).sub.2, and (GGGGS).sub.1 sequences, respectively, whereas the fourth was prepared by joining the V(H) and V(L) domains directly. Each construct was fused to a derivative of human IgG1 (hinge plus CH2 plus CH3) to facilitate purification using staphylococcal protein A. The

aggregation and CD20 binding properties of these four 1F5 scFv-Ig derivatives produced were investigated. Both size- exclusion HPLC column analysis and Western blots of proteins subjected to nonreducing SDS-PAGE suggested that all four 1F5 scFv-Ig were monomeric with m.w. of .sim.55 kDa. The CD20 binding properties of the four 1F5 scFv-Ig were studied by ELISA and flow cytometry. The 1F5 scFv-Ig with the 5-aa linker (GS1) demonstrated significantly superior binding to CD20-expressing target cells, compared with the other scFv-Ig constructs. Scatchard analysis of the radiolabeled monovalent GS1 scFv-Ig revealed a binding avidity of 1.35×10^{10} compared with an avidity of 7.56×10^8 for the native bivalent 1F5 Ab. These findings suggest that the GS1 scFv-Ig with a short linker peptide of .sim.5 aa is the best of the engineered constructs for future studies.

L6 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN

AN 1997:366666 CAPLUS

DN 127:14108

TI High protein production from exogenous gene in recombinant eukaryotic cells without amplification of exogenous gene

IN Dorai, Haimanti; Oppermann, Herrmann

PA Creative Biomolecules, Inc., USA

SO U.S., 24 pp., Cont. of U. S. Ser. No. 143,498, abandoned.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5631158	A	19970520	US 1995-461184	19950605
	US 5658763	A	19970819	US 1995-463675	19950605
	US 5733782	A	19980331	US 1995-464589	19950605
PRAI	US 1993-143498	B1	19931025		

AB Disclosed herein are improved methods and compns. for achieving enhanced protein prodn. expressed from non-native gene constructs, including single chain scFv and derivative sequences. The methods and compns. are particularly useful for creating stably transfected, constitutively expressing immortalized mammalian cell lines that exhibit high recombinant protein productivity while maintaining a low copy number per cell of the non-native recombinant DNA sequence encoding the protein of interest. Thus, an immortalized eukaryotic cell is multiply transfected with a gene of interest and at least one, and preferably two, expression effector genes of viral origin competent to effect expression of the non-native gene of interest, culturing the transfected cell under appropriate selection conditions such that the transfected DNA is stably integrated into the cell genome, and selecting a clone that expresses at least 1 µg protein/106 cells/mL. Plasmid pH1176, containing the adenovirus transcription transactivator E1A gene under control of the thymidine kinase promoter, plasmid pH1130 containing the adenovirus VA1 gene for translation stimulator RNA, and plasmid pH1512 encoding an scFv under control of the cytomegalovirus major immediate early promoter/enhancer were prepared. CHO cell clones transfected with these plasmids produced ≥6.5 µg protein/106 cells/mL.

L6 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN

AN 1998:25974 CAPLUS

DN 128:152928

TI A recombinant single chain antibody neutralizes coronavirus infectivity but only slightly delays lethal infection of mice

AU Lamarre, Alain; Yu, Mathilde W. N.; Chagnon, Fanny; Talbot, Pierre J.

CS Laboratory Neuroimmunovirology, Virology Research Center, Institut Armand-Frappier, Universite Quebec, Laval, QC, H7V 1B7, Can.

SO European Journal of Immunology (1997), 27(12), 3447-3455

CODEN: EJIMAF; ISSN: 0014-2980

PB Wiley-VCH Verlag GmbH

DT Journal

LA English

AB The variable region genes of a murine anti-coronavirus monoclonal antibody (mAb) were joined by assembly polymerase chain reaction and expressed in *Escherichia coli* in a single chain variable fragment (scFv) configuration. After induction of expression, the expected 32-kDa protein was identified by Western immunoblotting with specific rabbit anti-idiotypic antibodies. The scFv fragments were purified from soluble cytoplasmic preps. by affinity chromatog. on Ni agarose, which was possible with an N-terminal but not with a C-terminal His tag. Purified scFv fragments retained the antigen-binding properties of the parental antibody, could inhibit its binding to viral antigens with apparently higher efficiency than monovalent antigen-binding (Fab) fragments, but neutralized viral infectivity with lower efficiency (about 7-fold at a molar level). To evaluate the usefulness of these smaller and less immunogenic mols. in the treatment of viral diseases, mice were treated with purified recombinant scFv fragments and challenged with a lethal viral dose. A small delay in mortality was observed for the scFv-treated animals. Therefore, even though the scFv could neutralize viral infectivity in vitro, the same quantity of fragments that partially protected mice in the form of Fab only slightly delayed virus-induced lethality when injected as scFv fragments, probably because of a much faster in vivo clearance: the biol. half-life was estimated to be about 6 min. Since a scFv derived from a highly neutralizing and protective mAb is only marginally effective in the passive protection of mice from lethal viral infection, the use of such reagents for viral immunotherapy will require strategies to overcome stability limitations.

=> s VH and substitution and scFv and stability and production

L7 4 VH AND SUBSTITUTION AND SCFV AND STABILITY AND PRODUCTION

=> duplicate remove l7

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L8 4 DUPLICATE REMOVE L7 (0 DUPLICATES REMOVED)

=> d l8 bib abs 1-4

L8 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN

AN 2006:869401 CAPLUS

TI Optimizing the Generation of Recombinant Single-Chain Antibodies Against Placental Alkaline Phosphatase

AU Sheikholvaezin, Ali; Sandstroem, Per; Eriksson, David; Norgren, Niklas; Riklund, Katrine; Stigbrand, Torgny

CS Departments of Clinical Microbiology and Immunology, Umea University, Umea, Swed.

SO Hybridoma (2006), 25(4), 181-192

CODEN: HYBRAV; ISSN: 1554-0014

PB Mary Ann Liebert, Inc.

DT Journal

LA English

AB Recombinant technologies to engineer ordinary hybridoma monoclonal antibodies (MAbs) to single-chain fragment variable (scFv) may cause loss of antibody affinity, increased tendency to aggregate, increased temperature sensitivity, and low yield of active protein. In the present investigation, the well-characterized MAb H7 against placental alkaline phosphatase (PLAP), used as a model antibody, was engineered to improve solubility and stability of scFv with retained high affinity. The original procedure to generate single-chain antibodies with a 10-amino acid linker between VH and VL yielded an almost

insol. product. By site-directed mutagenesis, four selective sequence substitutions were made in the VL fragment and one in the VH fragment to improve solubility. The importance of the linker length was investigated, and a 25/30 amino acid linker was found to improve solubility. In order to further increase the stability of the single-chain antibody, an addnl. covalent -S-S- bond was introduced between amino acid 100 in the VL fragment and amino acid 44 in the VH region, to make a single-chain disulfide stabilized variable fragment (scdsFv). Altogether five different antibody constructs were produced and compared in terms of solubility, stability, affinity, and prodn. properties. Immunospecificity was tested by ELISA (ELISA) against the target antigen, temperature sensitivity by exposing the purified scFv to higher temps. All the new constructs retained almost equal activity and high affinity for their target antigen, placental alkaline phosphatase (PLAP), compared to the intact MAb H7, up to +42°C as evaluated by ELISA. The overall affinity $K_A > 10^9$ (M⁻¹) of the new antibodies could be maintained in the same order of magnitude as the original one (H7), when evaluated by Biacore technol. The best final single-chain antibody was obtained by performing the specific site-directed mutations and introducing a linker of 30 amino acids, but not by addnl. stabilizing disulfide bonds. The yield of the final antibody was improved approx. 10-fold by the modifications. This antibody could easily be expressed in a bacterial system using the PET-32a TrxA vector and the Escherichia coli strain BL21 Origami B (DE3). Purified antibody, which could be kept at concns. up to 0.8 mg/mL, was obtained, which is sufficient for clin. testing of therapeutic applications.

L8 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2005:546857 CAPLUS
DN 143:76819
TI Single chain Igs specific for various antigens including tumor and B cell antigens, recombinant production and immunological activities thereof
IN Ledbetter, Jeffrey A.; Hayden-Ledbetter, Martha; Thompson, Peter A.
PA USA
SO U.S. Pat. Appl. Publ., 338 pp., Cont.-in-part of U.S. Ser. No. 53,530.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 5

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2005136049	A1	20050623	US 2003-627556	20030726
	US 2003133939	A1	20030717	US 2002-53530	20020117
	CA 2533921	AA	20050224	CA 2003-2533921	20031224
	WO 2005017148	A1	20050224	WO 2003-US41600	20031224
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2003300092	A1	20050307	AU 2003-300092	20031224
	EP 1654358	A1	20060510	EP 2003-800349	20031224
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	BR 2003018417	A	20060725	BR 2003-18417	20031224
	CN 1852976	A	20061025	CN 2003-80110470	20031224
	NO 2006000764	A	20060420	NO 2006-764	20060217
PRAI	US 2001-367358P	P	20010117		

US 2002-53530 A2 20020117
US 2003-627556 A 20030726
WO 2003-US41600 W 20031224

AB The invention provides recombinant single chain antibodies (scFvs) composed of: (a) variable regions of heavy or light chain Igs that may contain a linker sequence; (b) hinge regions of Igs; and (c) CH2 and CH3 constant regions of Igs. Specifically, the invention relates said scFvs may contain: (a) wild-type or mutant/variant variable region of Igs, wherein amino acid substitutions lead to an increase in stability and/or expression of scFvs; (b) wild-type or mutant hinge regions of IgG, IgA or IgE isolated from various organisms that contain zero, one, or two cysteine residues; and (c) wild-type or mutant/truncated IgG or IgA. The invention also relates that said recombinant scFv possess a variable region that bind specific antigens, such as tumor antigens, B cells antigens or B cell differentiation antigens, and that said scFvs are capable of at least one immunol. activity, such as antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC). The invention further relates that said recombinant scFvs may be coupled to a drug, toxin, immunomodulator, label and/or effector moiety. The invention also provides approx. 103 scFv constructs generated from the following hybridomas: murine 2H7 (anti-human CD20), 4.4.220 (anti-human CD40), 2e12 (anti-human CD28), 10A8 (anti-human CD152/CTLA-4), G19-4 (anti-human CD3), L6 (anti-carcinoma), FC2-2 (anti-CD16), UCHL-1 (anti-CD45RO), HD37 (anti-CD19), G19-4 (anti-CD3), and 5B9 (anti-human 4-1BB/CD137), and rat 1D8 (anti-murine 4-1BB/CD137). In the examples, the invention described the recombinant prodn. of disclosed scFvs for various antigens. The sequences for various Ig regions used in construction of scFvs were presented. The immunol. activities of these scFvs were demonstrated.

L8 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2005-09844 BIOTECHDS
TI New non-naturally occurring single chain protein comprising polypeptides with binding domain, connecting regions and N-terminally truncated immunoglobulin, having immunological activity, useful for neutralizing infectious agent;

single chain protein production via plasmid expression in
host cell for use in neutralization of bacterium

AU LEDBETTER J A; HAYDEN-LEDBETTER M S; THOMPSON P A
PA TRUBION PHARM INC
PI WO 2005017148 24 Feb 2005
AI WO 2003-US41600 24 Dec 2003
PRAI US 2003-627556 26 Jul 2003; US 2003-627556 26 Jul 2003

DT Patent
LA English
OS WPI: 2005-182370 [19]
AN 2005-09844 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A non-naturally occurring single chain protein comprising first polypeptide with a binding domain comprising heavy chain variable region, second polypeptide comprising a connecting region attached to first polypeptide, and a third polypeptide comprising N-terminally truncated immunoglobulin heavy chain constant region polypeptide, where non-naturally occurring single-chain protein is capable of immunological activity, is new.

DETAILED DESCRIPTION - A non-naturally occurring single chain protein (I) comprises a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, the binding domain polypeptide comprising a heavy chain variable region, which comprises an amino acid substitution or deletion at one or more amino acid residues, a second polypeptide comprising a connecting region attached to the first polypeptide, and a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached

to the second polypeptide, where the non-naturally occurring single-chain protein is capable of an immunological activity. INDEPENDENT CLAIMS are also included for the following: (1) reducing a target cell population in a subject, involves administering to the subject a protein that is less than 150 kD, which involves treating the target cell population with a first protein or peptide that binds to cells within the target cell population, and treating the target cell population with a second protein or peptide that capable of at least one of binding an Fc receptor, inducing target cell apoptosis, or fix complements, where the first protein or peptide molecule is directly connected to the second protein or peptide molecule or, optionally, the first protein or peptide molecule and the second protein or peptide molecule are linked by a third protein or peptide molecule, and where the protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope; (2) depleting (M1) cells in an animal, involves administering a modified IgE protein into the blood stream of an animal; (3) a polynucleotide (II) that encodes (I); (4) a cell containing (II); (5) a recombinant vector capable of expressing (I); (6) expressing (I) under conditions in which (I) is expressed; (7) a composition comprising (I) in combination with one or more additional therapeutic compounds; (8) displaying recombinant molecules, which molecules include a native or engineered immunoglobulin heavy chain variable region, the improvement comprising an immunoglobulin heavy chain region that includes one or more mutation, substitution, alteration, and/or deletion at one or more amino acid residue corresponding to positions 9, 10, 11, 12, 108, 110, and 112 in the heavy chain variable region; and (9) a non-naturally occurring single chain antigen-binding protein comprising protein having a mutation chosen from 2H7 scFv VH L11S (CSC-S) H WCH2, WCH3, 2H7 scFv VH L11S IgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgAH WgACH2 T4CH3, 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11S (SSS-S) IT P331S CH2 WCH3, 2HQ scFv VH L11S (CSS-S) H P331S CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3, G28-1 scFv (SSS-S) H WCH2 WCH3, G28-1 scFv IgAH WCH2 WCH3, G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3, 2H7 scFv IgAH IgAGH2CH3, 2H7 scFv IgAH IgAHCH2 T18CH3, 2Hand-40.2.220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-ccd20-anti-cd40) 2H7 scFv IgAH IgACH2 T4CH3-hCD89 TM/CT, GI9-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT, 2el2 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT, etc.

BIOTECHNOLOGY - Preferred Protein: In (I), the binding domain polypeptide comprising a heavy chain variable region comprising one or more amino acid deletion or substitution in positions 9, 10, 11, 12, 108, 110, 112 and the protein has an increased recombinant expression or stability relative to the protein not having an amino acid deletion or substitution. (I) is capable of binding to the target molecule, antibody dependent cell-mediated cytotoxicity and complement fixation, and is capable of decreasing the number of target cells. (I) has an increased expression or stability in mammalian cells relative to a protein not having the amino acid substitution. The second polypeptide comprises an N-terminally truncated IgE immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, where the non-naturally occurring single-chain protein is capable of an immunological activity. The connecting region comprises first, second, and third cysteine residues, where the first cysteine residue is N-terminal to the second cysteine and the second cysteine is N-terminal to the third cysteine, where one or both of the second and third cysteine residues is substituted or deleted, and where the non-naturally occurring single-chain protein is capable of

an immunological activity. The amino acid deletion or substitution in the first polypeptide is at positions 12, 80, 81, 83, 105, 106, and 107. The connecting region comprises at least a portion of an IgA hinge region. The connecting region attached to the first polypeptide, comprises three cysteine residues and one proline residue, where one or more of the cysteine residues is deleted or substituted. The binding domain polypeptide is a single chain Fv. The one or more amino acid substitution or deletion in the heavy chain variable region is effective to increase expression or stability of the protein relative to a protein without the deletion or substitution. The binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide. (I) further comprises a second binding domain polypeptide capable of binding a second target molecule, the second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide. The first target molecule and the second target molecule are different or same. The binding domain polypeptide is a single chain Fv comprising an amino acid substitution at position 11 in the heavy chain variable region. The amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region is selected from serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine. The leucine at position of 11 of the single chain Fv heavy chain variable region is replaced with an amino acid other than serine or by serine at position 11, and, where the protein is capable of antibody dependent cell-mediated cytotoxicity and complement fixation, and is capable of binding to the target molecule decreasing a number of target cells. The leucine is replaced by des-leucine at position-11. (I) has an increased recombinant expression or stability relative to the protein not having an amino acid substitution at position 11. The expression of the protein having an amino acid substitution at position 11 is 10-100 fold greater than the protein without a substitution at position 11. The expression is in mammalian cells. The binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of the single chain Fv has been deleted. The binding domain polypeptide is a single chain Fv and the binding domain polypeptide comprises a light chain variable region, where the light chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107. The amino acid at position 106 has been substituted or deleted. The binding domain polypeptide binds to a tumor antigen. The binding domain polypeptide binds to an antigen on an immune effector cell. The binding domain polypeptide binds to a cancer cell antigen. The cancer cell antigen is a surface antigen. The cancer cell antigen is an intracellular antigen. The binding domain polypeptide binds to a B cell antigen. The B cell antigen is selected from CD19, CD20, CD22, CD37, CD40, CD80, and CD86. The single chain Fv binds to a B cell antigen. The single chain Fv is selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv. The single chain Fv is selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, IDS, and 4.4.220 single chain Fv. The binding domain polypeptide is an scFv that binds to a B cell differentiation antigen. The binding domain polypeptide binds to a target selected from CD2, CD3, CD4, CD5, CD6, CD8, CD10, GDI Ib, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II. The protein is capable of forming a complex comprising two or more of the proteins. The complex is a dimer or monomer. (I) is coupled to a drug, toxin, immunomodulator, polypeptide effector, isotope, label, or effector moiety. The immunological activity is selected from antibody dependent cell-mediated

cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin. The immunological activity comprises two immunological activities selected from antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin. (I) is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components, or which is capable of induction of one or more immune effector cells selected from NK cells, monocytes, macrophages, B cells, T cells, mast cells, neutrophils, eosinophils, and basophils. The induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules. (I) is capable of cellular activation, where the activation leads to changes in cellular transcriptional activity. The cellular transcriptional activity is increased or decreased. The one or more biologically active molecules is a protease or cytokine. The cytokine is selected from moriokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins. (I) is capable of neutralization of an infectious agent, where the infectious agent is a bacterium, a virus, a parasite, or a fungus. (I) is capable of neutralization of a toxin, where the toxin is selected from endotoxins and exotoxins. The exotoxin is selected from anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, Escherichia coli heat-labile toxin LT, E.coli heat stable toxin ST, shiga toxin Pseudomonas exotoxin A, botulinum toxin, tetanus toxin, Bordetella pertussis AC toxin, and Bacillus anthracis EF. The toxin is an endotoxin selected from saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins. (I) is capable of binding to an intracellular target to effect a cellular function. The binding domain polypeptide comprises a light chain variable region attached to the heavy chain variable region by a binding domain linker, where the binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser. (I) comprises three Gly-Gly-Gly-Gly-Ser peptides. The binding domain polypeptide comprises wild-type or engineered immunoglobulin variable region obtained from species selected from human, murine, rat, pig, and monkey. The binding domain polypeptide comprises a humanized immunoglobulin variable region. The N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide. The binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region. The connecting region comprises a naturally occurring hinge region selected from a human hinge or its portion, human IgG hinge or its portion, human IgA hinge or its portion, human IgE hinge or its portion, camelid hinge region or its portion, IgG1 llama hinge region or its portion, nurse shark hinge region or its portion, and spotted ratfish hinge region or its portion. The connecting region preferably comprises a human IgE hinge or its portion. The connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue. The connecting region comprises a human IgGA hinge region having between zero and two cysteine residues. The connecting region comprises a wild-type human IgG1 immunoglobulin hinge region. The connecting region comprises a glycosylation site. The connecting region has no cysteine residues capable of forming disulfide bonds or has one cysteine residue. The connecting region comprises a mutated wild-type immunoglobulin hinge

region polypeptide comprising not more than, one cysteine residue. The connecting region is altered such that the protein has a reduced ability to dimerize. The connecting region comprises three cysteine residues and one proline residue, where one or more of the cysteine residues is deleted or substituted and the proline residue is substituted or deleted. The wild-type hinge region polypeptide is from human IgG1. The heavy chain constant region of the connecting region comprises CH2 and CH3 domains from IgG1, where proline is replaced by serine at position 331 in the CH2 region, threonine is replaced by asparagines at position 256, lysine is replaced by glutamine at position 290, glutamic acid is replaced by lysine at position 258, and alanine is replaced by proline at position 339 in the CH2 region. Preferred Methods: In (M1), the modified IgE protein is administered or coadministered with a histamine release blocker. The connecting region comprises a IgG hinge or its portion, and the heavy chain constant region is from IgE and comprises CH3 and CH4 domains without a CH2 domain. The single chain protein comprises a single chain Fv binding domain from a 2e12, and the heavy chain constant region comprises IgE CH2, CH3, and CH4 domains, and where the heavy chain constant region is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

ACTIVITY - Antibacterial; Virucide; Antiparasitic; Fungicide. No supporting data is given.

MECHANISM OF ACTION - None given.

USE - (I) is useful for neutralization of an infectious agent, where the infectious agent is a bacterium, a virus, a parasite, or a fungus (claimed).

EXAMPLE - No relevant example is given. (590 pages)

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TI Two amino acid mutations in an anti-human CD3 single chain Fv antibody fragment that affect the yield on bacterial secretion but not the affinity

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AB Recombinant antibody fragments directed against cell surface antigens have facilitated the development of novel therapeutic agents. As a first step in the creation of cytotoxic immunoconjugates, the authors constructed a single-chain Fv fragment derived from the murine hybridoma OKT3, that recognizes an epitope on the ϵ -subunit of the human CD3 complex. Two amino acid residues were identified that are critical for the high level prodn. of this scFv in Escherichia coli. First, the substitution of glutamic acid encoded by a PCR primer at position 6 of VH framework 1 by glutamine led to a more than a 30-fold increase in the prodn. of soluble scFv. Second, the substitution of cysteine by a serine in the middle of CDR-H3 addnl. doubled the yield of soluble antibody fragment without any adverse effect on its affinity for the CD3 antigen. The double mutant scFv (Q,S) proved to be very stable in vitro: no loss of activity was observed after storage for 1 mo at 4°, while the activity of scFv containing a cysteine residue in CDR-H3 decreased by more than half. The results of prodn. yield, affinity, stability measurements and anal. of three-dimensional models of the structure suggest that the sixth amino acid influences the correct folding of the VH domain, presumably by affecting a folding intermediate, but has no effect on antigen binding.

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